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ESR Characterization of a Co(II)–Tetraphenylporphyrin–Thiolate Complex and Its Oxygen Adduct. A Model of Cytochrome P-450 Related Heme Proteins

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This paper reports the first successful example of the detection and characterization of 5-coordinate Co(II)–tetraphenylporphyrin–thiolate (Co(II)-TPP–S$^-$) and 6-coordinate Co(II)TPP–S$^-$(O$_2$) complexes by ESR spectroscopy. The ESR parameters characterized for these new complexes are akin to those reported both for the cobalt substituted cytochrome P-450cam and its dioxygen adduct [1].

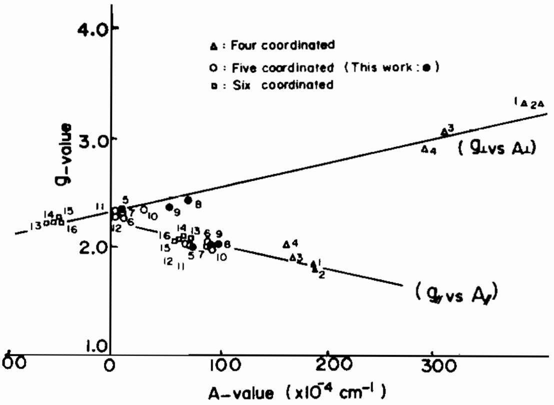

Fig. 1. The relationship between the observed g- and A-tensors as plotted for Co(II)–porphyrin complexes with different axial coordination. 1: Co(II)TPP in CHCl$_3$ [4]; 2: Co(II)TPP in H$_2$TPP [4]; 3: Co(II)TPP in Co(III)TPP(Cl) [2]; 4: Co(II)–tetra(*p*-methylphenyl)porphyrin, [(*p*-CH$_3$)-TPP], in toluene [3]; 5: Co(II)TPP–pyridine; 6: Co(II)–myoglobin [5]; 7: Co(II)–hemoglobin [6]; 8: Co(II)TPP–SH(TGE); 9: Co(II)TPP–S$^-$(TGE); 10: Co(II)–P-450cam [1]; 11: Co(II)–P-450cam with camphor [1]; 12: Co(II)–tetra(*p*-methoxyphenyl)porphyrin, [(*p*-OCH$_3$)TPP], –(hexylamine)$_2$ [3]; 13: Co(II)(*p*-OCH$_3$)TPP–(pyridine)$_2$ [3]; 14: Co(II)(*p*-CH$_3$)TPP–(picoline)$_2$ [3]; 15: Co(II)–protoporphyrin IX dimethylester–(pyridine)$_2$ [6]; 16: Co(II)–(*p*-OCH$_3$)TPP–(picoline)$_2$ [3].

Thiol ligands (SH) used are thioglycolic acid (TG) esters; TG-ethyl (TGE), -n-butyl (TGB), -iso-octyl (TGO) and -ethyl-hexyl esters (TGEH). The samples were prepared in an anaerobic ESR tube connected to three glass tubes at room temperature and the ESR spectra were measured at 77 K. Unoxygenated Co(II)TPP–SH complex was prepared by mixing

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TABLE I. ESR parameters of deoxygenated Co(II)TPP-thiol and -thiolate complexes.

Axial ligand	Thiol (SH)				Thiolate (S$^-$)			
	$g_\perp$	$g_\parallel$	$A_\perp$ cm^{-1} x 10^{-4} (Gauss)	$A_\parallel$	$g_\perp$	$g_\parallel$	$A_\perp$ cm^{-1} x 10^{-4} (Gauss)	$A_\parallel$
TGE	2.36	2.02	52.9	98.5	2.33	2.02	29.4	91.3
			(47.9)	(104.5)			(27.0)	(97.0)
TGB	2.36	2.02	59.3	98.4	2.35	2.03	31.3	88.7
			(53.9)	(104.5)			(28.6)	(93.5)
TGO	2.36	2.02	56.9	98.5	2.33	2.02	29.4	92.1
			(51.7)	(104.5)			(27.0)	(94.6)
TGEH	2.36	2.03	54.0	99.0	2.33	2.02	28.2	92.3
			(49.0)	(105.1)			(25.9)	(97.9)
Propanethiol	2.41	2.03	56.0	94.8				
			(50)	(100)				
Cobalt-P450cam					2.32	2.03	2	67.3
							(2.0)	(71)
Cobalt-P450cam with camphor					2.31	2.03	2	69.1
							(2.0)	(73)

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TABLE II. ESR parameters of oxygenated Co(II)TPP-thiol, thiolate and those of free superoxide.

Axial ligand	Thiol (SH)				Thiolate (S^-)				Free O_2^-	
	$g_\perp$	$g_\parallel$	$A_\perp$ cm^{-1} × 10^{-4} (Gauss)	$A_\parallel$	$g_\perp$	$g_\parallel$	$A_\perp$ cm^{-1} × 10^{-4} (Gauss)	$A_\parallel$	$g_\perp$	$g_\parallel$
TGE	1.990	2.083	10.9 (11.7)	23.1 (23.8)	2.003	2.089	9.7 (10.4)	14.0 (14.4)	2.007	2.093
TGB	1.991	2.081	10.4 (11.2)	22.7 (23.4)	2.003	2.089	9.7 (10.4)	14.2 (14.6)	2.007	2.092
TGO	1.992	2.083	10.6 (11.4)	22.9 (23.5)	2.004	2.090	9.6 (10.3)	14.3 (14.7)	2.007	2.092
TGEH	1.991	2.082	10.8 (11.6)	22.7 (23.4)	2.005	2.090	9.6 (10.3)	14.4 (14.8)	2.006	2.094
Propanethiol	1.96	2.02	12.9 (14.0)	17.2 (18.2)						
Cobalt P450cam						2.067		18.8 (19.5)		
Cobalt-P450cam with camphor					2.008	2.079		15.0 (15.5)		

Co(II)TPP solution (1 mM in CH_2Cl_2, 0.5 ml) with thiol compound (0.5 M in acetone, 0.02 ml) *in vacuo*. Unoxygenated Co(II)TPP–S^- complex was obtained by mixing Co(II)TPP solution (1 mM in CH_2Cl_2, 0.5 ml) with the solution composed of thiol compound (0.5 M in acetone, 0.01 ml) and Me_4NOH (1 M in methanol, 0.01 ml) *in vacuo*. To prepare the oxygen adducts, Co(II)TPP–SH(O_2) and Co(II)TPP–S^-(O_2), the samples were exposed to air for 30 sec. A free superoxide was prepared by aeration for over 3 min of unoxygenated Co(II)TPP–S^- solution, which contained twice the amount of thiol and Me_4NOH.

The observed g- and A-values of thiol and thiolate complexes prepared *in vacuo* for TGE (Table I) are plotted on the linear relationship established between the g- and A-tensors of low spin Co(II) porphyrin complexes with different axial coordination numbers (Fig. 1) [2]. The ESR parameters characterized as unoxygenated thiol and thiolate complexes exhibit an excellent fit on the plot as defined by 5-coordinate Co(II) complexes with a square pyramidal geometry.

It is known that all the values of $A_\parallel$, $A_\perp$ and $g_\perp$ can be affected by the strength of the axial donor in the 5-coordinate Co(II) complexes with low spin $3d_{z^2}$ ground state [3, 7]. Usually the axial ligand with a strong donor shows a decrease in the $A_\parallel$, $A_\perp$ and $g_\perp$ values with poorly resolved cobalt hyperfine structures (hfs) in the perpendicular regions. This effect is evident for the ESR parameters of cobalt substituted cytochrome P-450cam [1], which shows a large decrease in $A_\parallel$, $A_\perp$ and $g_\perp$ values compared with those of several Co(II)TPP–SH complexes (Table I). Reduction in the $A_\parallel$, $A_\perp$ and $g_\perp$ values for the thiolate model complexes is consistent with the trend seen for the cobalt substituted cytochrome P-450cam [1].

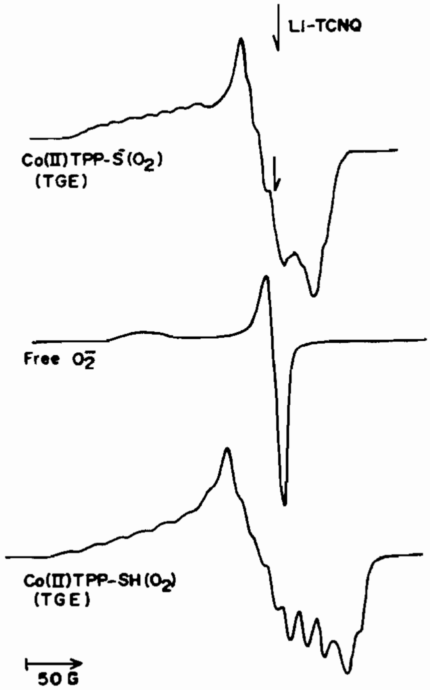

Fig. 2. ESR spectra of Co(II)TPP–S^-(O_2), Co(II)TPP–SH(O_2) and a free superoxide, O_2^-.

The ESR spectra of 6-coordinate dioxygen adducts of Co(II)–porphyrins are substantially different from those of the deoxy complexes. They exhibit greatly reduced cobalt hfs with slightly rhombic g-tensors, g_z g_x g_y [8–10]. The ESR parameters of O_2-adducts are typically reported in terms of a pseudo-axial symmetry, with $g_\parallel = g_z > g_\perp = (g_x + g_y)/2$ and $A_\parallel = A_z, A_\perp = (A_x + A_y)/2$ [11–14].

The ESR spectra recorded for oxygenated Co(II)-TPP–SH, Co(II)TPP–S^- and free superoxide systems are illustrated in Fig. 2. The ESR spectrum of Co(II)-TPP–TGE–$S^-(O_2)$ exhibits rhombic g and A anisotropies which can be assigned to those reported for 6-coordinate dioxygen adducts [14]. The total width of each spectrum decreases in the following order: oxygenated Co(II)TPP–SH > oxygenated Co(II)TPP–S^- > free superoxide. In fact, a significant reduction in $A_\parallel$ value can be seen on replacing the axial ligand from thiol to thiolate.

The ESR parameters of oxygenated thiol and thiolate complexes and of a free superoxide are summarized. Based on the results of Table II, the following important features of the cobalt–dioxygen bond perturbed by the axial thiolate can be speculated. (1) Both $A_\parallel$ and $A_\perp$ values measured for thiolate complexes are consistently smaller than those of thiol and pyridine ($g_\perp$ = 1.994, $g_\parallel$ = 2.072, $A_\perp$ = 11.5, $A_\parallel$ = 15.8, A: $cm^{-1} \times 10^4$) [3] complexes. (2) The g-factors of thiolate complexes are all akin to that of a free superoxide. In particular, the $g_\perp$ values of thiolate complexes are close to that of a free spin, g_e, while those of thiol, pyridine [3] and cobalt substituted heme proteins with N-donors [15, 16] are always lower than g_e. The smaller values of $A_\parallel$ and $A_\perp$ as characterized for thiolate complexes imply that a lesser extent of unpaired delocalization would be expected between the cobalt–dioxygen bonding in the presence of the axial thiolate donor. This means that the cobalt–dioxygen bonding nature in Co(II)–TPP–S^- is predominantly ionic in character.

In terms of the molecular orbital description [9, 10, 17], a contribution to the g-factors due to the metal angular momentum resulting from a mixing of metal d- and dioxygen π-orbital can be effectively deduced in this case, thus the observed g-factors reveal a concomitant approach to that of a free superoxide. The observed trend of the ESR parameters characterized by the present model complexes is also valid for the oxygenated cobalt cytochrome P-450cam [1]. The $g_\perp$ value reported exceeds slightly the g_e value and the $A_\parallel$ value is relatively small.

A successful identification of Co(II)TPP–$S^-(O_2)$, as demonstrated in the present chemical model, is of great significance not only in justifying the validity of the reaction scheme previously proposed [18], but also in mimicking the process of dioxygen activation in cytochrome P-450 related heme proteins.

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